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Immunochromatographic Detection of Canine Distemper Virus as an Evaluation of Cross-Species Transmissions in Wildlife of Sepilok, Sabah

By

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A research paper submitted in partial fulfillment of the requirement for the degree of Doctor of Veterinary Medicine

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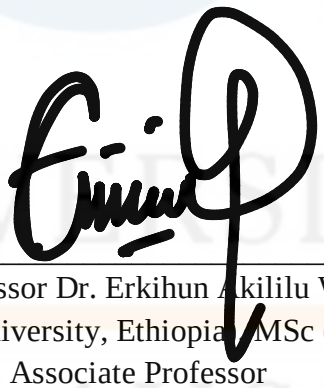

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ABSTRACT

Canine distemper virus (CDV) is a highly contagious and potentially fatal RNA virus belonging to the *Morbillivius* family. Previously notorious for infecting Canidae and Felidae, recent reports in wildlife have sparked its threat on their conservation. With the objective of determining its prevalence in free-ranging and captive animals in Kabili-Sepilok Forest Reserve, this study measured the seropositivity of CDV antibodies in six animal species in Sabah, Malaysia, including captive Bornean sun bears, captive Bornean orangutans, captive Bornean elephants, free-ranging macaques, stray dogs, and wild rats. Canine distemper antibody seropositivity and titre were detected using Bionote Anigen Rapid CDV Ab Test Kit 2.0 using whole blood samples. Of the 90 animals tested, a total of 91.11% were seropositive to canine morbillivirus antibodies. The findings of this study highlighted the potential risks of cross-species transmission and emphasised the need for integrated disease surveillance and conservation efforts to protect threatened wildlife species.

Keywords: Borneo, canine distemper virus, canine morbillivirus, cross-species, wildlife.

ABSTRAK

Virus distemper kanin (CDV) ialah virus RNA yang sangat mudah berjangkit dan berpotensi membawa maut, tergolong dalam keluarga Morbillivirus. Sebelum ini terkenal kerana menjangkiti spesies Canidae dan Felidae, laporan terkini mengenai jangkitan pada hidupan liar telah menimbulkan ancaman terhadap pemuliharaan mereka. Dengan objektif untuk menentukan kelaziman virus ini dalam hidupan liar dalam kurungan dan Hutan Simpan Kabili-Sepilok, kajian ini mengukur seropositiviti antibodi CDV dalam enam spesies haiwan di Sabah, Malaysia, termasuk beruang madu Borneo dalam kurungan, orang utan Borneo dalam kurungan, gajah Borneo dalam kurungan, kera liar, anjing terbiar, dan tikus liar. Kehadiran antibodi Canine Distemper dan titre antibodi dikesan menggunakan Kit Ujian Pantas Bionote Anigen CDV Ab 2.0 melalui sampel darah penuh. Daripada 90 haiwan yang diuji, sebanyak 91.11% menunjukkan seropositiviti terhadap antibodi canine morbillivirus. Penemuan kajian ini menekankan potensi risiko penularan antara spesies dan menegaskan keperluan untuk pemantauan penyakit yang bersepadu serta usaha pemuliharaan bagi melindungi spesies hidupan liar yang terancam.

Kata Kunci: Borneo, hidupan liar, penularan silang spesies, virus distemper kanin, virus morbillivirus anjing.

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Airi

Family

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DEDICATION

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LIST OF ABBREVIATIONS

Ab	Antibody
BSBCC	Bornean Sun Bear Conservation Centre
CD	Canine Distemper
CDV	Canine Distemper Virus
CNS	Central Nervous System
IUCN	International Union for Conservation of Nature
Ml	Millilitres
Ml	Microlitre
RNA	Ribonucleic Acid
RDC	Rainforest Discovery Centre
SLAM	Signalling Lymphocytic Activation Molecular
SORC	Sepilok Orangutan Rehabilitation Centre
VN	Virus Neutralisation

CHAPTER 1

INTRODUCTION

Canine morbillivirus (CDV), formerly known as canine distemper virus (ICTV, 2023), is a pantropic, enveloped RNA virus belonging to the genus *Morbillivirus* and the family Paramyxoviridae (Martella et al., 2008). The CDV is predominant in Canidae and Felidae, in which it is known to cause severe immunosuppression, respiratory signs, and neurological lesions (Beineke et al., 2015). However, more recent reports indicated outbreaks among a wider host range, including raccoon (*Procyon lotor*), Eurasian badger (*Meles meles*), Asian black bear (*Ursus thibetanus*), long-tailed macaques (*Macaca fascicularis*), binturong (*Arctictis binturong*) and Asian elephant (*Elephas maximus*), which could be a source of infection for domestic dogs and other wildlife species (Oni et al., 2006; Beineke et al., 2015; Rein-Weston, 2023).

CDV in the wildlife of Malaysia was not given much attention until it was detected in Malayan tigers (Ten, et al, 2019). However, detection of positive cases remains low, possibly due to underdiagnosis and lack of appropriate testing approaches in the field.

The Bornean Sun Bear Conservation Centre (BSBCC) and Sepilok Orangutan Rehabilitation Centre (SORC) are two wildlife conservation centres situated at the Kabili-Sepilok Forest Reserve (43.0 km^2), neighbouring one another. BSBCC is an NGO-based conservation centre specifically for Bornean sun bears; whereas SORC is a government-based centre for the conservation of Bornean Orangutan as well as other rescued wildlife in Sabah. Both facilities work closely for the benefit of local wildlife. Currently, BSBCC has 43 rescued sun bears in captivity, whereas SORC has approximately 20 captive Bornean orangutans. (Bornean Sun Bear Conservation Centre, 2014; Orangutan Appeal UK, 2017).

The rescued sun bears and orangutans have outdoor access as part of the rehabilitation process, sharing the same forest environment with other free-ranging wildlife such as macaques, rats, civets, tree shrews, squirrels, etc. The free-ranging animals feed on the food remnants provided to the sun bears and orangutans. It is also common for direct contact between macaques and orangutans or sun bears during feeding time. (Bornean Sun Bear Conservation Centre, 2014; Orangutan Appeal UK, 2017). The close interaction of captive sun bears or orangutans with other wild mammals poses an intricate risk of disease transmission. Stray dogs surrounding the forest edge at the car park or housing area can also serve as the source for shared pathogens (Martinez-Gutierrez & Ruiz-Saenz, 2016).

In late 2023, a total of four sun bears at BSBCC were noticed to exhibit neurological signs resembling canine morbillivirus infection, and disease onset for the affected bears was several months apart. Additionally, they came from different age groups, sexes, social groups, and forest enclosures. Thus, CDV antibody test kits were used to test the affected animals and others in the vicinity. All ten animals tested, including seven sun bears, and one rat were positive; while one orangutan and one elephant tested were negative. However, bear samples submitted for PCR analysis and serum neutralisation tests turned out to be negative for canine morbillivirus (Yeoh, pers. comm.). Although CDV was ruled out as the cause of the neurological signs, establishing the prevalence of potential pathogens remains crucial to safeguard these protected species, especially given the lack of diagnostic tests specifically designed for wildlife and the potential for CDV to mutate, leading to genetic variants that may not be detectable with standard tests designed for domestic dogs (Seki et al., 2022).

1.1 Problem Statement

For CDV virus to be maintained in a population in an enzootic state, the population must be large enough to produce a continuous source of susceptible animals. Hence, this study aimed to focus CDV detection in free-ranging rats and macaques, besides the signature animals at BSBCC and SORC, the sun bear, orangutan, and elephants. This condition is similar to that reported in Europe, where free-ranging animals were able to sustain the transmission of CDV (Beineke et al., 2015). By gaining more information about the CDV situation at both BSBCC and SORC, it was expected to help formulate appropriate control and prevention measures to curb the spread of CDV in the animal population. Bornean sun bears (*Helarctos malayanus euryspilus*) are listed as vulnerable, while Bornean orangutans (*Pongo pygmaeus*) are critically endangered under the IUCN Red List (Husson et al., 2016; *The IUCN Red List of Threatened Species*, 2019). Thus, it is imperative that actions be taken to protect the animals in captivity and surrounding natural habitat from fatal diseases such as canine distemper.

1.2 Research Question

What is the seropositivity of CDV antibodies in sun bears, orangutans, macaques, elephants, stray dogs, and rats surrounding BSBCC and SORC?

1.3 Research Hypothesis

Free-ranging animals around BSBCC and SORC act as seropositive carriers and possible shedders of canine morbillivirus to facilitate its transmission across species.

1.4 Research Objectives

a. To determine the prevalence of canine distemper (CD) in free-ranging animals (macaques, rats, and stray dogs) around Bornean Sun Bear Conservation Centre (BSBCC) and Sepilok Orangutan Rehabilitation Centre (SORC).

b. To ascertain the prevalence of CD in captive wildlife (Bornean sun bears, Bornean orangutan and Bornean elephant) at BSBCC and SORC.

c. To investigate the potential transmission of CDV from free-ranging animals to captive wildlife at BSBCC and SORC.

1.5 Significance of Study

This research was the first detection of CDV antibodies in a diverse range of species in the same environment in Malaysia. It is also the first reported detection of CDV antibodies in Bornean elephants, Bornean orangutans and wild rats in Malaysia. Hence, this research highlights the interconnectedness of domestic and wildlife populations in disease transmission. The findings of this research aimed to provide a critical baseline data, allowing for targeted control and prevention strategies to safeguard these species. Beyond conservation, this study underscores the importance of integrated disease surveillance and its role in protecting biodiversity, particularly in ecologically sensitive areas such as Sabah. It hopes to contribute to the broader understanding of cross-species transmission of CDV in Southeast Asia.

1.6 Scope of Study

The study was conducted in Sepilok, Sandakan, Malaysian Borneo. The samples were collected during July 2024 to October 2024 from BSBCC, SORC and its vicinity within the Kabili-Sepilok Forest Reserve.

CHAPTER 2

LITERATURE REVIEW

2.1 Viral Properties and Pathogenesis

CDV is an enveloped single-stranded RNA (ssRNA) virus with a negative sense genome (Loots et al., 2017). The virus possesses several critical proteins that mediate its pathogenesis: the haemagglutinin (H) and fusion (F) proteins facilitate viral entry and fusion with host cells, while the matrix (M) protein plays a key role in virus assembly and budding. The virus also includes two polymerase-associated proteins, phosphoprotein (P) and large protein (L), which are essential for replication and transcription. Additionally, the nucleocapsid protein (N) encapsulates the viral RNA, contributing to viral stability and protection (Duque-Valencia et al., 2019).

CDV is primarily transmitted through aerosol droplets, excretions, and oral route, with predilection towards the respiratory epithelium (Beineke et al., 2015). The virus binds to nectin-4 and the Signaling Lymphocytic Activating Molecular (SLAM) protein of macrophages and lymphocytes (Duque-Valencia et al., 2019). This receptor-binding facilitates viral entry into host cells, initiating a cascade of immunological disruptions.

Once inside the host, CDV targets macrophages and lymphocytes, triggering apoptosis and severe immunosuppression. This immunosuppressive effect hampers the host's ability to mount effective immune responses, predisposing the host to secondary infections and contributing to the systemic spread of the virus via the lymphatic system (Beineke et al., 2015). As the virus disseminates through the haematological system and cerebrospinal fluid, it can cause widespread lesions, particularly affecting the central nervous system (CNS). It shows a strong predilection for astrocytes, where it induces demyelination and neurologic symptoms

such as seizures and ataxia (Carvalho et al., 2012). This neural infection may lead to severe, often fatal outcomes, especially in species with limited immune response.

The gastrointestinal tract is also frequently affected, with lesions ranging from mild inflammation to severe necrotizing enteritis, contributing to the high mortality seen in acute outbreaks, particularly in immunologically naïve populations such as wildlife species (Loots et al., 2017). Interestingly, some species, including felids, can exhibit fatal CDV infections with little to no clinical signs, making diagnosis and early intervention challenging (Beineke et al., 2015). This silent progression emphasizes the virus's ability to evade early detection and underscores the importance of monitoring at-risk populations.

Despite its high mortality in many species, CDV has a single serotype, but multiple genotypes exist, with polymorphisms most notably in the H protein, which may affect its transmissibility and virulence. Understanding these genetic variations is crucial for assessing the virus's potential for cross-species transmission and for the development of vaccines that can protect a broader range of susceptible species (Loots et al., 2017).

2.2 Role of Reservoirs in CDV Epidemiology

CDV transmission between domestic dogs and wildlife depends on the dynamics between their populations, genetic characteristics of the virus, host receptors, and other factors that are not fully understood (Duque-Valencia et al., 2020; Olarte-Castillo et al., 2013; Sarute & Ruiz-Sáenz, 2016). Although the initial spread of CDV resulting from interactions between domestic canids and wildlife has been shown to trigger high mortality in wildlife, CDV spreading from wildlife to domestic canids is also possible (Kapil & Yeary, 2011; Olarte-Castillo et al., 2013). An example of such dynamics was observed in the Serengeti ecosystem, where domestic dog populations were initially identified as the source of CDV outbreaks in lions. However, over time, the outbreaks in lions became asynchronous with

those in domestic dogs, suggesting the presence of other wildlife reservoirs maintaining the virus in the ecosystem (Craft et al., 2008; Beineke et al., 2015). Similar patterns have been reported in other regions, where species like raccoons and hyenas act as secondary reservoirs, facilitating the virus's persistence and transmission across diverse habitats (Duque-Valencia et al., 2019; Rein-Weston, 2023). The bidirectional nature of this transmission is particularly concerning, as wildlife reservoirs not only suffer significant population impacts but may also spill the virus back into domestic animal populations, exacerbating outbreak dynamics.

2.3 Cross-species Transmission

Historically coined as a disease affecting Canidae only, CDV has now been known to affect Felidae, Mustelidae, Procyonidae, Ursidae, Viverridae, Cricetidae, Cercopithecidae, Suidae and Elephantidae (Duque-Valencia et al., 2019). Although over 98 species have been known to be susceptible to CDV, it is undoubtedly underreported due to the lack of data from free-ranging wildlife (Rein-Weston, 2023). As stated by Beineke et al., "*Spillover of CDV resulting from interactions between domestic or feral dogs and various wild species has led to mass mortalities in several wildlife species, but also spillback events from wildlife reservoir hosts to domesticated animals occur*."

The broad host range of CDV is a result of its evolutionary adaptation, stemming from its common ancestor with rinderpest virus. Rinderpest, which primarily infects cloven-hoofed animals, is believed to have evolved into CDV, adapting to domestic canids before spilling over into wildlife populations (Quintero-Gil et al., 2023). This evolutionary process likely led to the emergence of Phocine Distemper Virus (PDV) responsible for affecting marine mammals, which has caused significant outbreaks in seals and other marine species (Miller et al., 2009). The transition of CDV from terrestrial mammals to

marine species highlights its capacity for cross-species transmission, with its continued evolution in wildlife reservoirs increasing the likelihood of further spillover events. (Quintero-Gil et al., 2023).

2.4 Threat to Wildlife Conservation

In the 1980s, it was believed that Felids remained highly immune to CDV due to lower SLAM binding affinity and high CDV-neutralizing antibodies which rendered them immune or asymptomatic despite repeated exposure to infected Canids (Beineke et al., 2015). This belief was cemented with the exposure of African lions (*Panthera leo*) to CDV-infected jackals through two epidemics in the Serengeti National Park, Tanzania. However, in 1994, a third CDV epidemic caused severe encephalitis and pneumonia, killing 30% of the entire Serengeti lion population. (Roelke-Parker et al., 1996). Home to the largest remaining population of African lions worldwide, an incurable fatal disease in Tanzania can cause a drastic decline in the world population of African lions. What is more, this outbreak spread to wildlife in Maasai Mara National Park, Kenya, to several other species such as hyenas (*Crocuta crocuta*), leopards (*Panthera pardus*) and bat-eared foxes (*Otocyon megalotis*) in the vicinity, causing high morbidity and mortality. (Packer et al., 2010).

2.5 Zoonotic Potential

Despite no confirmed natural cases of CDV infection in humans, experimental studies have demonstrated the potential zoonotic risk. Monkeys induced with a modified CDV strain, CYN07-dV, which had slight alterations to its H protein, were successfully infected when the virus was introduced to human SLAM CD150 and nectin-4 receptors (Beineke et al., 2015). SLAM receptor of humans and non-human primates share 86% of similarity (Duque-Valencia

et al., 2019). This genetic similarity raises concerns, as it could facilitate CDV's ability to infect humans, especially those unvaccinated against measles virus (*Morbillivirus hominis*) (Beineke et al., 2015; Duque-Valencia et al., 2019; Quintero-Gil et al., 2019). Additionally, CDV's high mutation rate and genetic adaptability further heighten concerns about the virus's potential to evolve and infect humans, particularly in environments where humans come into frequent contact with wildlife or domestic animals. While no natural human infections have been observed, the possibility of a zoonotic strain emerging remains a threat, emphasizing the importance of vigilant monitoring of CDV in susceptible animal populations in order to protect public health (Quintero-Gil et al., 2019).

CHAPTER 3

RESEARCH METHODOLOGY

This research was conducted on 90 animals of six different species (Table 3.1) in BSBCC, SORC and its surrounding forest enclosure in Sabah, Borneo. Both BSBCC and SORC are located within the Kabili-Sepilok Forest Reserve, a protected area for wildlife within close proximity to human settlement. Whole blood from these animals were collected and tested for CDV antibodies using an immunochromatographic test kit.

Table 3.1: List of Animals Sampled for CDV Antibodies

No.	Common Name	Scientific Name	Quantity
1.	Bornean Sun Bear	*Helarctos malayanus euryspilus*	14
2.	Bornean Orangutan	*Pongo pygmaeus*	20
3.	Long-tailed Macaque	*Macaca fascicularis*	3
4.	Pig-tailed Macaque	*Macaca nemestrina*	3
5.	Bornean Elephant	*Elephas maximus borneensis*	6
6.	Domestic Dog	*Canis lupus familiaris*	14
7.	Rat	*Rattus sp.*	25
Total Samples			90

All sun bears sampled were captive animals at BSBCC, and all orangutans and elephants sampled were captive animals at SORC. Other animal species are free-ranged wildlife and strays found within and adjacent areas to BSBCC and SORC.

3.1 Sample Collection

3.1.1 Sun Bear

Sun bears were anaesthetised with xylazine 1 mg/kg in combination with zolazepam and tiletamine 3 mg/kg administered intramuscularly using blow dart for health examination or clinical treatment. Once the bear reached anaesthesia stage three plane two, they were transferred from the enclosure to Sepilok Wildlife Clinic on a stretcher via a van. Site of the blood collection was shaved and disinfected with 70% alcohol. Approximately 0.5 ml of blood was withdrawn using a 3 ml hand syringe with a 23-gauge hypodermic needle from the cephalic vein. After transferring back to their enclosure, anaesthesia was reversed with yohimbine 0.15 mg/kg using a 1 ml hand syringe with a 23-gauge hypodermic needle. No post-anaesthetic complications were observed.

3.1.2 Orangutan

Captive orangutans were physically restrained for blood collection, without need for chemical restraint as they were trained for blood collection through operant conditioning. Site of the blood collection was disinfected with 70% alcohol. Approximately 0.5 ml of blood was withdrawn using a 3 ml hand syringe with 23 gauge hypodermic needle from the cephalic vein.

3.1.3 Macaque

Macaques in the forest surrounding SORC/BSBCC were trapped in a geotextile fabric trap (Figure. 3.1) lured with banana bait. Macaques within the SORC compound were trapped using a stainless-steel trap (Figure. 3.1) lured with banana bait. They were then darted with xylazine

1 mg/kg and ketamine 10 mg/kg, once anaesthetised they were transferred to Sepilok Wildlife Clinic for sampling. Site of the blood collection was prepared by shaving off the fur and disinfection with 70% alcohol. Approximately 0.5 ml of blood was withdrawn using a 3 ml hand syringe with a 23-gauge hypodermic needle for adult macaques, and a 1 ml hand syringe with 25-gauge hypodermic needle for juvenile macaques, from cephalic, femoral or saphenous vein. After sampling, they were released back to their respective capture sites in the forest.

Figure 3.1

a. Macaque Forest Trap **b. Macaque Metal Trap**

3.1.4 Elephant

Bornean elephants rescued by the Sabah Wildlife Department kept at SORC, were physically restrained with rope by their keeper for blood sampling. Site of sampling was disinfected with 70% alcohol before collection. Approximately 0.5 ml of blood was collected from the auricular vein using a 3 ml hand syringe with an 18 gauge hypodermic needle.

3.1.5 Stray Dog

Six of the 14 stray dogs were captured from the Sepilok residential area for the Sepilok Trap, Neuter and Release (TNR) program. The dogs were darted with xylazine 1 mg/kg and ketamine 10 mg/kg intramuscular via manual blow dart or gun dart. The sedated dog was then placed in

a stainless-steel metal cage and transferred to the neutering room near Rainforest Discovery Centre (RDC) for the neutering procedure.

While another three of the 14 stray dogs, being of fairly calm demeanour, were physically restrained and sampled in the location they were found in. One hand was used to restrain their head while the other hand occluded their forelimb for sampling, and a muzzle was used to secure the jaw. These dogs were conditioned with dog biscuits two to three times daily for at least one week preceding the sampling. Site of blood collection disinfected with a 70% alcohol swab. Approximately 0.3 ml of blood was collected from the cephalic vein using a 1 ml hand syringe with a 23-gauge hypodermic needle.

3.1.6 Rat

Rats were caught by placing small mammal traps in areas suspected to have interspecies contact between rats and captive wildlife at BSBCC and SORC. A total of 25 traps were used, out of which 10 traps were measuring 28 cm by 14 cm and the remaining 15 traps were measuring 18 cm by 10 cm. Locations of placement included forest area shared by BSBCC and SORC, near sun bear and orangutan feeding sites respectively. Traps placed in the forest were tied to adjacent tree using a raffia string to prevent damage from free-roaming macaques (Figure 3.3). One end of the string was tied to the trap in a noose knot, while the other end was tied to the tree in a square or overhand knot depending on applicability The cranial end of the trap was kept elevated atop a branch or two to facilitate closure of the trap door upon ingestion of the bait. Plant shoots were placed upright around the trap, and leaves were placed flat on top of the trap for camouflage while keeping in mind to not create excessive weight that might have prevented the trap door from closing. It was also kept in mind to place the forest traps away from areas with a high population of green pit vipers. Accidental captures included three tree shrews and one lone skink lizard which were released unharmed.

Figure 3.3: Rat Trap Set Up in Forest

Baits used for the traps were banana, maize, and either of those covered with peanut butter. Success rate of rats trapped was highest for maize covered in peanut butter. Using maize over banana also increased its durability and reduced likelihood of the bait being stolen by macaques. Banana baits were replaced daily, while corn baits were replaced once every two days. All set traps were checked for animals at least twice daily.

The trapped rats were relocated to Sepilok Wildlife Clinic for sampling, where they were physically restrained with rope technique. The rope used was an approximately 30 cm long raffia string with a noose knot. The noose knot was inserted through the trap bar (figure 4) , and the rat was manoeuvred into the noose using a wooden branch stick with its end covered in peanut butter. Once the rat's cranial body including head and forelimbs were within the noose, the rope was gently tightened to restrain for sedation with xylazine 1 mg/kg intramuscularly, using a 3 ml hand syringe with 25-gauge needle inserted through the openings in the trap. Care was taken to ensure the rope remained loose enough to not restrict breathing. Once sedated, the rope was removed and the rat was physically restrained with a gloved hand.

The rat's head was secured between the restrainer's thumb and index fingers while remaining fingers were used to support the chest. A 1 ml hand syringe with 25-gauge hypodermic needle was used to withdraw approximately 0.3 ml blood intracardially. Being pests, the rats were then euthanized with pentobarbital 50 mg/kg injected intraperitoneally using a hand syringe with 3 ml 25-gauge hypodermic needle.

Figure 3.4: Rat Physical Restraint

Rat carcasses were buried in a 5-metre-deep pit in the forest, to minimise soil contamination with pentobarbital residue and to prevent scavenging by free-roaming wildlife. Site of burial was covered with dried leaves to further deter scavengers away.

3.2 Sample Processing

Samples collected in Sepilok Wildlife Centre were immediately processed in their laboratory. Samples collected on site (6 elephants and 3 stray dogs) were stored in an EDTA tube, transported to the laboratory, and processed all within less than 30 minutes. All samples were tested for CDV using Bionote Anigen Rapid CDV Ab Test Kit 2.0 (Bionote Inc, Hwaseong-si, Korea).

Firstly, one drop of whole blood was placed on a specimen sheet. An inverted cup was used to retrieve 5 µL and transferred to the assay diluent tube. four drops of the whole blood and

diluent mixture was transferred to the immunochromatographic test kit using a disposable dropper. Results were observed and recorded after 10 minutes.

3.3 Result Interpretation

Only one line at control ("C") was interpreted as a negative result. Absence of control ("C") line was interpreted as an invalid result. Colour development at control ("C") and test ("T") was interpreted as a positive result, and further divided into antibody titre. Colour development at test ("T)" lighter than colour development at control ("C"), was interpreted as low antibody titre. Colour development at test ("T") same as colour development at control ("C") was interpreted as medium antibody titre. Colour development at test ("T") darker than colour development at control ("C") was interpreted as high antibody titre. Colour grade scale was used to confirm results and avoid subjective bias. This is summarised in the table below (Table 3.2).

Table 3.2: Bionote Anigen Rapid CDV Ab Test Kit 2.0 Result Interpretation

Result	Interpretation	Antibody Titre	VN* Titre	Colour Scale
C line	Negative	-	-	-
No C line	Invalid	-	-	-
T line < C line	Positive	Low	1:4	1-2
T line = C line		Medium	1:8	3
T line > C line		High	1:16	4-5

**VN - Virus Neutralisation Test*

CHAPTER 4

RESULTS

4.1 Detection of CDV Antibodies

From the 90 animals sampled for CDV antibodies, 91.11% were positive, and 8.89% were negative. Positive results per species were as follows (Figure 4.1): 94.74% of Sun Bears, 95.00% of Orangutans, 100.00% of Macaques, 100.00% of Elephants, 92.86% of Dogs, and 80.00% of Rats.

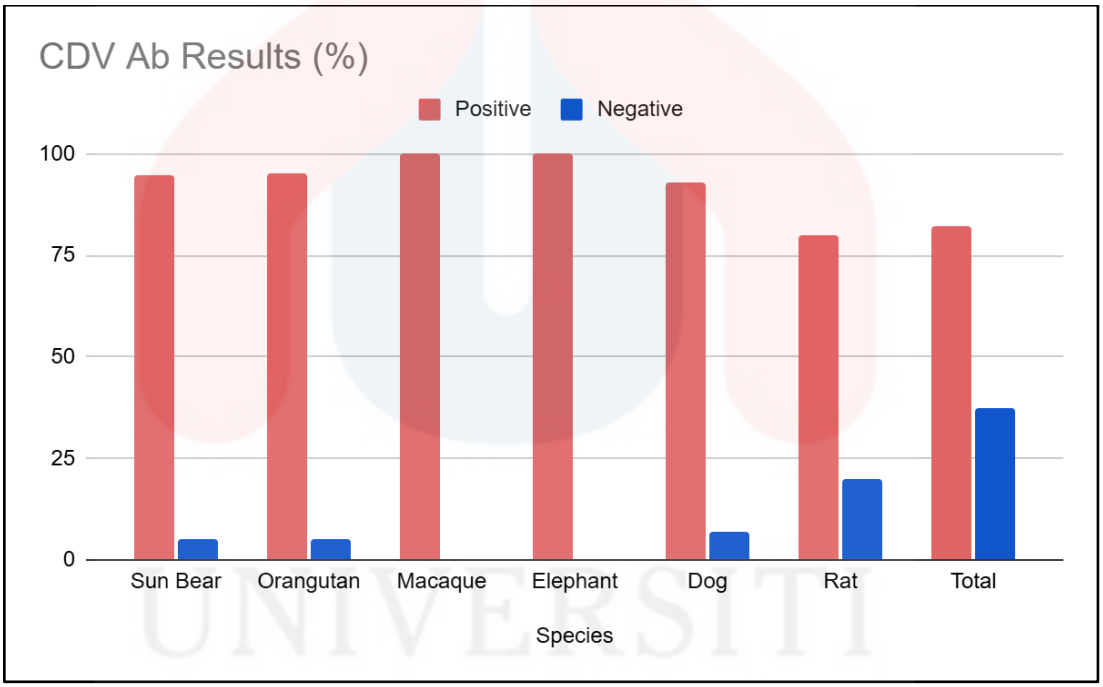

Figure 4.1. Results per Species

Antibody titers per species are tabulated below in Table 4.1.

Table 4.1. Result Summary

Result Summary					
Species	Quantity	Positive			Negative
		Low	Medium	High	
Sun Bear	19	18			1
Orangutan	20	5	3	11	1
Macaque	6	4	1	1	
Elephant	6	6			
Dog	14	9	4		1
Rat	25	14	2	4	5
Total	90	82			8

4.2 Statistical Analysis

Table 4.2. Chi-Square Analysis

	Value	Degree of Freedom	Asymptotic Significance (2-sided)
Pearson Chi-Square	994.386	12	<.001
Likelihood Ratio	598.369	12	<.001
No. of Valid Cases	935		

A chi-square test of independence was conducted using IBM SPSS Statistics 27 software to assess the association between the presence of canine distemper virus (CDV) antibodies in six different species. The results of Pearson chi-square analysis revealed a chi-square value of 994.386 and a p-value of 0.000 ($p < 0.05$), suggesting there is a highly significant association between the species and their CDV results. The likelihood ratio test further supports this finding, with a chi-square value of 598.369 and a p-value of 0.000 ($p < 0.05$).

CHAPTER 5

DISCUSSION

5.1 Possible Cross-Species Transmission

Seropositivity to CDV antibodies in all tested macaques, as well as the majority of the tested wild rats and stray dogs, supported the hypothesis that free-ranging animals acted as reservoirs, potentially transmitting the virus to captive wildlife. Since stray dogs and wild rats shared the same forest enclosure with significant movement across the habitat, it is highly likely that free-ranging macaques could get infected and act as reservoirs. Given that primates and wild rats have close interactions with the habitat, saliva from cross contamination of food, or aerosolized droplets, could make transmission of CDV feasible even in the absence of overt clinical signs. This mode of transmission, known as "silent circulation," could have led to the production of antibodies in the captive wildlife, suggesting possible subclinical or mild infection. Several studies had shown that, even without clinical symptoms, animals could produce antibodies in response to CDV exposure (Hernandez et al., 2020; Haas et al., 2021).

5.2 Antibody Titre Level

In this study, some rats that were trapped and tested were observed to be less than 1.5 weeks of age, which may not have been sufficient time for the development of antibodies against CDV. Antibody production generally requires time for an immune response to occur, typically taking several days to weeks post-exposure (Stuart et al., 2015). Young animals, particularly those under 2 weeks old, may not have fully matured immune systems capable of mounting a significant response to viral infections, which could explain the lack of detectable antibodies in certain individuals.

Additionally, it was noted that all five rats that tested negative for CDV antibodies, as well as a few with low antibody titres, appeared to be younger. This finding is consistent with previous studies that have suggested younger animals, especially those not yet fully immunocompetent, may not demonstrate robust antibody responses (Crawford et al., 2019). The age of the animal is a crucial factor influencing immune response, with older animals typically exhibiting more pronounced antibody production compared to younger ones.

Moreover, the methodology used in this study involved whole blood sampling, which may have contributed to the lower observed antibody titres. Whole blood contains a lower concentration of antibodies compared to serum due to the presence of other cellular components such as erythrocytes, leukocytes and thrombocytes (Kurtz et al., 2010). This means that an animal with low or medium antibody titres in whole blood could potentially exhibit higher titres if serum had been tested instead. Previous studies have demonstrated that serum samples typically offer more precise and higher antibody concentrations than whole blood samples (Hernandez et al., 2017).

5.3 Possible Cross Reactivity

The immunochromatographic assay used in this study, traditionally used to test for CDV in Canids, is known to cross-react with other morbilliviruses such as rinderpest virus (RPV), peste des petits ruminants virus (PPRV), and measles virus (Beineke et al., 2015; WOAH, 2018). While RPV has been eradicated globally and PPRV is not commonly found in the species tested, non-human primates may have residual immunity against measles, leading to possible cross-reactivity (Cattadori et al., 2017). For wild rats, other morbilliviruses, such as Porcine Respiratory Virus (PRV) could also contribute to cross-reactivity (Geisbert et al., 2017).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In conclusion, the immunochromatographic assay used to detect antibodies against CDV in the whole blood of Bornean sun bears, Bornean elephants, Bornean orangutans, long-tailed macaques, pig-tailed macaques, stray dogs, and wild rats, revealed a 91.11% positivity rate among the 90 animals sampled. These findings support the hypothesis that free-ranging wildlife may act as a reservoir for canine distemper virus, potentially leading to sporadic outbreaks in captive wildlife and posing a risk of mutation that could drastically impact conservation efforts.

However, as this study is preliminary, further research is crucial to establish a comprehensive baseline for understanding and mitigating the risk of outbreaks in vulnerable or critically endangered wildlife populations. Future investigations should account for factors such as age uniformity in each species and employ serum samples for more precise antibody titre measurements. Additionally, serum neutralisation tests or PCR analysis of organs from post-mortem of wild rats could provide critical data for confirming the role of free-ranging animals as reservoirs.

Outside of Asia, CDV has already caused catastrophic declines, with some wildlife populations facing near extinction. This serves as a stark warning that similar outbreaks could threaten other species, including those in protected environments. The ability of CDV to infect human cells in laboratory settings further intensifies concerns, as it highlights the growing risk of mutation. Should this virus continue to evolve, it has the potential to emerge as a zoonotic crisis, posing a significant threat to both wildlife conservation and human health. Immediate action is crucial, as failure to address this looming risk could result in a new pandemic with far-reaching consequences.

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APPENDICES

Appendix 1. RAW Data

Canine Distemper Virus Antibody RTK Results							
No.	Location	Species	Animal ID	Blood Collection		Test Date	Results
				Date	**Route**		
1	Other*	Dog	TNR51, near RDC	31/7/2024	Cephalic	31/7/2024	Scale 3-4
2	Other*	Dog	TNR52, near RDC	31/7/2024	Cephalic	31/7/2024	Scale 3-4
3	Other*	Dog	TNR53, near SORC	31/7/2024	Cephalic	31/7/2024	Scale 3-4
4	SORC	Orangutan	OSPIE	1/8/2024	Cephalic	1/8/2024	Scale 5-6
5	SORC	Orangutan	CINTA	1/8/2024	Cephalic	1/8/2024	Scale 1-2
6	SORC	Orangutan	HUJAN	1/8/2024	Cephalic	1/8/2024	Scale 1-2
7	SORC	Orangutan	OYO	1/8/2024	Cephalic	1/8/2024	Scale 1-2
8	SORC	Orangutan	AGOP	1/8/2024	Cephalic	1/8/2024	Scale 1-2
9	SORC	Orangutan	ADIE	1/8/2024	Cephalic	1/8/2024	Scale 1-2
10	SORC	Orangutan	MODA	1/8/2024	Cephalic	1/8/2024	Scale 5-6
11	SORC	Orangutan	NAPAGANG	1/8/2024	Cephalic	1/8/2024	Negative
12	SORC	Orangutan	BARYL	1/8/2024	Cephalic	1/8/2024	Scale 1-2
13	SORC	Orangutan	BEGIA	2/8/2024	Cephalic	2/8/2024	Scale 3-4
14	SORC	Orangutan	MICHELLE	2/8/2024	Cephalic	2/8/2024	Scale 5-6
15	SORC	Orangutan	TOMBILINA	2/8/2024	Cephalic	2/8/2024	Scale 5-6
16	SORC	Orangutan	NAMI	2/8/2024	Cephalic	2/8/2024	Scale 5-6
17	SORC	Orangutan	BAKUT	2/8/2024	Cephalic	2/8/2024	Scale 3-4
18	SORC	Orangutan	TOMBIRUO	2/8/2024	Cephalic	2/8/2024	Scale 5-6
19	SORC	Orangutan	LUFFY	2/8/2024	Cephalic	2/8/2024	Scale 1-2
20	SORC	Orangutan	ARCHIE	2/8/2024	Cephalic	2/8/2024	Scale 3-4
21	SORC	Orangutan	CERAH	2/8/2024	Cephalic	2/8/2024	Scale 5-6
22	SORC	Orangutan	WULAN	2/8/2024	Cephalic	2/8/2024	Scale 5-6

23	SORC	Orangutan	ROSA	2/8/2024	Cephalic	2/8/2024	Scale 5-6
24	BSBCC	Sun Bear	LINGGAM	14/8/2024	Cephalic	14/8/2024	Scale 1-2
25	BSBCC	Sun Bear	AH BUI	14/8/2024	Cephalic	14/8/2024	Scale 1-2
26	BSBCC	Sun Bear	ITAM	15/8/2024	Cephalic	15/8/2024	Scale 1-2
27	BSBCC	Sun Bear	WAN WAN	16/8/2024	Cephalic	16/8/2024	Scale 1-2
28	BSBCC	Sun Bear	MAMATAI	16/82024	Cephalic	16/8/2024	Scale 1-2
29	BSBCC	Sun Bear	KUAMUT	15/8/2024	Cephalic	15/8/2024	Scale 1-2
30	BSBCC	Sun Bear	FULUNG	22/8/2024	Cephalic	22/8/2024	Scale 1-2
31	BSBCC	Sun Bear	OM	22/8/2024	Cephalic	22/8/2024	Scale 1-2
32	BSBCC	Sun Bear	CHIN	23/8/2024	Cephalic	23/8/2024	Scale 1-2
33	BSBCC	Sun Bear	KUKUTON	23/8/2024	Cephalic	23/8/2024	Negative
34	BSBCC	Sun Bear	AH LUN	26/8/2024	Cephalic	26/8/2024	Scale 1-2
35	BSBCC	Sun Bear	SOO	26/8/2024	Cephalic	26/8/2024	Scale 1-2
36	BSBCC	Sun Bear	SIGALUNG	27/8/2024	Cephalic	27/8/2024	Scale 1-2
37	SORC	Rat	RAT 1	2/9/2024	Tail	2/9/2024	Negative
38	BSBCC	Rat	RAT 2	6/9/2024	Tail	6/9/2024	Scale 5-6
39	SORC	Rat	RAT 3	11/9/2024	Intracardiac	11/9/2024	Scale 1-2
40	SORC	Rat	RAT 4	11/9/2024	Intracardiac	11/9/2024	Scale 1-2
41	SORC	Rat	RAT 5	11/9/2024	Intracardiac	11/9/2024	Scale 3-4
42	Other*	Macaque	MACAQUE 1	11/9/2024	Femoral	11/9/2024	Scale 1-2
43	SORC	Rat	RAT 6	12/9/2024	Intracardiac	12/9/2024	Negative
44*	SORC	Elephant	SULI	12/9/2024	Auricular	12/9/2024	Scale 1-2
45	SORC	Elephant	BENY	12/9/2024	Auricular	12/9/2024	Scale 1-2
46	SORC	Elephant	BRUMAS	12/9/2024	Auricular	12/9/2024	Scale 1-2
47	SORC	Elephant	SAHABAT	12/9/2024	Auricular	12/9/2024	Scale 1-2
48	SORC	Elephant	TABURI	12/9/2024	Auricular	12/9/2024	Scale 1-2
49	SORC	Elephant	YURI	12/9/2024	Auricular	12/9/2024	Scale 1-2
50	BSBCC	Rat	RAT 7	12/9/2024	Intracardiac	12/9/2024	Scale 1-2
51	BSBCC	Rat	RAT 8	12/9/2024	Intracardiac	12/9/2024	Scale 1-2
52	Other*	Macaque	MACAQUE 2	12/9/2024	Femoral	12/9/2024	Scale 3-4

53	Other*	Macaque	MACAQUE 3	12/9/2024	Femoral	12/9/2024	Scale 1-2
54	SORC	Rat	RAT 9	15/9/2024	Intracardiac	15/9/2024	Negative
55	SORC	Rat	RAT 10	15/9/2024	Intracardiac	15/9/2024	Scale 1-2
56	SORC	Rat	RAT 11	15/9/2024	Intracardiac	15/9/2024	Scale 1-2
57	SORC	Rat	RAT 12	15/9/2024	Intracardiac	15/9/2024	Negative
58	SORC	Rat	RAT 13	15/9/2024	Intracardiac	15/9/2024	Scale 1-2
59	SORC	Rat	RAT 14	15/9/2024	Intracardiac	15/9/2024	Scale 3-4
60	BSBCC	Rat	RAT 15	15/9/2024	Intracardiac	15/9/2024	Scale 1-2
61	BSBCC	Rat	RAT 16	15/9/2024	Intracardiac	15/9/2024	Scale 1-2
62	SORC	Rat	RAT 17	16/9/2024	Intracardiac	16/9/2024	Negative
63	BSBCC	Rat	RAT 18	17/9/2024	Intracardiac	17/9/2024	Scale 1-2
64	BSBCC	Rat	RAT 19	17/9/2024	Intracardiac	17/9/2024	Scale 1-2
65	BSBCC	Rat	RAT 20	17/9/2024	Intracardiac	17/9/2024	Scale 1-2
66	Other*	Dog	TNR54	18/9/2024	Cephalic	18/9/2024	Scale 3-4
67	Other*	Dog	TNR55	18/09/2024	Cephalic	18/9/2024	Scale 1-2
68	BSBCC	Sun Bear	PANDA	20/09/2024	Cephalic	20/09/2024	Scale 1-2
69	BSBCC	Rat	RAT 21	21/09/2024	Intracardiac	21/09/2024	Scale 1-2
70	SORC	Rat	RAT 22	21/09/2024	Intracardiac	21/09/2024	Scale 5-6
71	BSBCC	Rat	RAT 23	24/09/2024	Intracardiac	24/09/2024	Scale 1-2
72	BSBCC	Rat	RAT 24	24/09/2024	Intracardiac	24/09/2024	Scale 5-6
73	Other*	Macaque	MACAQUE 4	24/09/2024	Cephalic	24/09/2024	Scale 1-2
74	Other*	Macaque	MACAQUE 5	24/09/2024	Saphenous	24/09/2024	Scale 5-6
75	Other*	Dog	DOG 6	25/09/2024	Cephalic	25/09/2024	Scale 1-2
76	Other*	Dog	DOG 7	25/09/2024	Cephalic	25/09/2024	Scale 1-2
77	Other*	Dog	TNR56	25/09/2024	Cephalic	25/09/2024	Scale 1-2
78	Other*	Dog	DOG 9	25/09/2024	Cephalic	25/09/2024	Negative
79	Other*	Dog	TNR57	26/09/2024	Cephalic	26/09/2024	Scale 1-2
80	Other*	Dog	TNR58	26/09/2024	Cephalic	26/09/2024	Scale 1-2
81	Other*	Dog	TNR59	26/09/2024	Cephalic	26/09/2024	Scale 1-2
82	Other*	Dog	TNR60	26/09/2024	Cephalic	26/09/2024	Scale 1-2
83	SORC	Macaque	MACAQUE 6	29/09/2024	Saphenous	29/09/2024	Scale 1-2

84	SORC	Rat	RAT 25	29/09/2024	Intracardiac	29/09/2024	Scale 5-6
85	SORC	Dog	DOG 14	30/09/2024	Cephalic	30/09/2024	Scale 1-2
86	BSBCC	Sun Bear	MANIS	03/10/2024	Cephalic	03/10/2024	Scale 1-2
87	BSBCC	Sun Bear	BERMUDA	03/10/2024	Cephalic	03/10/2024	Scale 1-2
88	BSBCC	Sun Bear	PHIA	03/10/2024	Cephalic	03/10/2024	Scale 1-2
89	BSBCC	Sun Bear	JULAINI	04/10/2024	Cephalic	04/10/2024	Scale 1-2
90	BSBCC	Sun Bear	SUSIE	04/10/2024	Cephalic	04/10/2024	Scale 1-2

Appendix 2. UMK Animal Ethics Approval

UNIVERSITI MALAYSIA KELANTAN
Kampus Kota, 16100 Kota Bharu, Kelantan, Malaysia.
www.umk.edu.my Tel : 09-7717000/7281

FAKULTI PERUBATAN VETERINAR
Faculty of Veterinary Medicine

UMK/FPV/ACUE/FYP/006/2024
Ruj. Kami (*Our Ref.*) :
Tarikh (*Date*) : 11th August 2024

ASSOC. PROF. DR. CHOONG SIEW SHEAN
Main Supervisor
Faculty of Veterinary Medicine
University Malaysia Kelantan

Dear Assoc. Prof.,

APPROVAL OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) TO CONDUCT RESEARCH INVOLVING ANIMALS

We are pleased to inform you that your application for approval to conduct research from Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University Malaysia Kelantan has approved. Please refer the table below for approval code:

APPROVAL CODE	*UMK/FPV/ACUE/FYP/006/2024*
TITLE	IMMUNOCHROMATOGRAPHIC DETECTION OF CANINE DISTEMPER VIRUS AS AN EVALUATION OF CROSS-SPECIES TRANSMISSION FOR WILDLIFE IN SEPILOK, SABAH
NAME OF STUDENT	AISHATH AZLA

You are advised to always follow "3R" (REDUCE, REFINE, & REPLACE) and all animal ethics and animal welfare principles to reduce suffering in animal.

Thank you.

"ISLAM MEMBIMBING, RAJA MEMIMPIN, NEGERI DIBERKATI"
"MALAYSIA MADANI"
"BERKHIDMAT UNTUK NEGARA"

Yours sincerely,

(DR. MOHAMMED DAUDA GONI)
Chairman
Institutional Animal Care and Use Committee
Faculty of Veterinary Medicine

Appendix 3. Sabah Wildlife Department Permit

JABATAN HIDUPAN LIAR
(IBU PEJABAT)
WILDLIFE DEPARTMENT (HQ)
Tingkat 5, Blok B, Wisma MUIS,
5th Floor, Block B, MUIS Complex,
88100 KOTA KINABALU, SABAH, MALAYSIA.
No. Tel.: 088-215353, 210385, 215330, 213502, 214515, 215167, 215140.
No. Faks: 088-222476. E-mail: jhlsabah@tm.net.my
Sabah.Net: jhl@sabah.gov.my

(Sila catatkan Rujukan Fail kami ini apabila menjawab)

No. Ruj.: JHL.HQ.600-6/1
Tarikh: 02 Ogos 2024

Dekan
Fakulti Perubatan Veterinar
Universiti Malaysia Kelantan
Kampus Kota 16100 Kota Bharu, Kelantan, Malaysia
Email: shean.cs@umk.edu.my

Prof. Madya Dr.,

PERMOHONAN KEBENARAN DAN SURAT SOKONGAN PENYELIDIKAN HIDUPAN LIAR BAGI PROJEK AKHIR

Dengan merujuk surat Prof. Madya Dr. dengan No. Ruj.: UMK.A06.600-12/1/1 (11) bertarikh 04 Julai 2024.

i. Jabatan tiada halangan dan menyokong projek penyelidikan dengan butiran berikut:

No	Butiran Projek dan Kajian	Penyelidik	Penyelia
1	Immunochromatographic Detection of Canine Distemper Virus (CDV) as an Evaluation of Cross-species Transmission for Wildlife in Sepilok, Sabah	Azla Aishanth (No. Matrik: D20A0049)	i. Assoc. Prof. Dr. Choong Siew Shean ii. Dr. Nabila binti Sarkawi

Sekian, terima kasih.

"MALAYSIA MADANI"
"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

(SITI NUR'AIN AMPUAN ACHEH)
b.p Pengarah
Jabatan Hidupan Liar
SABAH

s.k. Assoc. Prof. Dr. Choong Siew Shean
email: shean.cs@umk.edu.my
s.k. Dr. Nabila binti Sarkawi
email: nabilasarkawi@gmail.com

"SABAH MAJU JAYA"

P.K. 0593 (L) - 2021

Appendix 4. Sabah Forestry Department Permit

JABATAN PERHUTANAN SABAH
(SABAH FORESTRY DEPARTMENT)
Laman Web (*Website*): www.forest.sabah.gov.my

Ibu Pejabat Perhutanan
(Forestry Headquarters)
Km 11, Jalan Utara
Beg Berkunci *(Locked Bag)* 68
90009, Sandakan
Sabah, Malaysia
Tel: +6089-242500
Fax : +6089-671303/672579

Sila catat rujukan kami apabila menjawab surat ini. *(Please quote this reference in any reply to this letter).*

Ruj. Tuan:
(Your Ref.)

Ruj. Kami:
(Our Ref.) **JPHTN/PSH 100-14/18/2/JLD.25(05)**

20th NOVEMBER 2024

Deputy Dean (Academic)
Faculty of Veterinary Medicine,
Universiti Malaysia Kelantan,
Kampus Kota,
16100 KOTA BHARU

Sir,

APPLICATION FOR PERMISSION TO CONDUCT FINAL YEAR PROJECT IN FOREST RESERVE AREAS

I was directed to refer your letter to the Chief Conservator of Forests with reference no. UMK.A06.600-12/1/1 (14) dated 10th November 2024 on the above-mentioned matter.

Please be informed that the department has no objection to your application to conduct research within the forest reserve, as per the following details:

Research Title:	Immunochromatographic Detection of Canine Distemper Virus as and Evaluation of Cross-species Transmission for Wildlife in Sepilok, Sabah	
Researcher:	1. Aishath Azla 2. Assoc. Professor Dr. Sandie Choong Siew Shean (Supervisor) 3. Assoc. Professor Dr. Erkiun Akililu Woldegiorgis (Co-supervisor) 4. Dr. Wong Siew Te, Bornean Sun Bear Conservation Centre (Local collaborator)	
Location under Sabah Forestry Department Jurisdiction:	**Location**	**District Forestry Office**
	Kabili-Sepilok FR (Class VI)	Sandakan
Duration:	November 2024 – November 2025	

'SABAH MAJU JAYA'
KAMI MENYASARKAN MENJADI YANG TERBAIK
WE AIM TO BE THE BEST

P.K. 0150 (L) - 2024

However, please take note that this permission is subject to the following conditions:

a) Obtain the Access License to conduct research in the State of Sabah from the Sabah Biodiversity Centre at the following address:-

Secretary of the Sabah Biodiversity Council,
Natural Resources Office,
Chief Minister's Department
19th Floor, Block A,
Sabah State Administrative Building
88400 KOTA KINABALU
Tel: 088-369000; Fax: 088-250753
Email: sabc@sabah.gov.my

b) Report to Sandakan District Forestry Officer (DFO) before entering the forest reserve, for operational and safety purposes, as per the following address:-

Forest Reserve Location	Forestry Officer In-Charge	Address/ Telephone No.
Kabili-Sepilok FR (Class VI)	Mr. Rosli Siki	Sandakan District Forestry Office P.O.Box 212, 90702 SANDAKAN Tel: 089 - 213966 / Fax: 089 - 213908

c) Settlement of permit to enter the forest reserve and other charges at any of our offices as mentioned above, as follows:-
   a. Permit to enter forest reserve: RM 5.00 / person/day/forest reserve;
   b. Private vehicles will be charged: RM 100 / vehicle at one time;
   c. Any other services required as per Annex 1.

d) Specimen collections (if any) are only for small quantities and are permitted for research purposes only;

e) Cutting off trees and plants is strictly prohibited;

f) To report the progress of research/activity **annually** to the Sabah Forestry Department and to be verified by DFO and Forest Research Centre (FRC).

g) One copy of the research report and a copy of the data collected must be handed over to the Sabah Forestry Department upon completion of the research work for record purposes; and

h) Sabah Forestry Department will not be held responsible for any untoward incidents or injuries during the research work conducted in the forest reserve.

Thank you very much.

"MALAYSIA MADANI"

"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

ZULKIFLI SUARA
for CHIEF CONSERVATOR OF FORESTS

cc. Secretary of the Sabah Biodiversity Council
Natural Resources Office,
Chief Minister's Department
19th Floor, Block A,
Sabah State Administrative Building
88400 KOTA KINABALU
Fax: 088-250753

For your information.
Thank you.

ZS/RA/ML/ml

Appendix 5. Sabah Biodiversity Centre Access License

Telephone: +6088 369370/ +6088369099
Facsimile : +6088 250753
Email address: sabc.sabah@gmail.com/
sabc@sabah.gov.my
Website URL: www.sabc.sabah.gov.my

(Please quote your licence reference number and date if you have any queries)

SABAH BIODIVERSITY COUNCIL
(Majlis Biodiversiti Sabah)
c/o Sabah Biodiversity Centre (SaBC)
Chief Minister's Department, 19th Floor,
Block A, Menara Kinabalu,
88400 Kota Kinabalu
SABAH, MALAYSIA

LICENCE REF.NO. : JKM/MBS.1000-2/2 JLD. 20 (145)

DATE OF APPROVAL : 06 NOVEMBER 2024

ACCESS LICENCE

(Section 8B(c) & 15 of Sabah Biodiversity Enactment 2000)

This is to certify that:
CHOONG SIEW SHEAN

Passport No./ MyKad No.:
750618-07-5454

of
Universiti Malaysia Kelantan (LC: Nabila Sarkawi, SWD / Wong Siew Te, BSBCC)

is authorized to access the following biological resources from the place(s) specified below, for **ACADEMIC PURPOSES**, upon terms and conditions hereinafter stipulated:

Research Title: IMMUNOCHROMATOGRAPHIC DETECTION OF CANINE DISTEMPER VIRUS AS AN EVALUATION OF CROSS-SPECIES TRANSMISSION FOR WILDLIFE IN SEPILOK, SABAH

No.	Common name	Scientific name	Descriptions (amount/number/volume)	Place(s) where access is permitted
1.	Sun bear	*Helarctos malayanus*	Intravenous blood collection through cephalic/femoral vein from 20 individuals per species. Less than 1% of their blood volume will be taken. This will be done under anesthesia to minimise stress.	Bornean Sun Bear Conservation Centre, Sepilok Orangutan Rehabilitation Centre **(BSBCC, Sabah Wildlife Dept., Sabah Forestry Dept.)**
2.	Orangutan	*Pongo pygmaeus*		
3.	Macaque	*Macaca sp.*	Intravenous blood collection through femoral vein from 40 individuals. Less than 1% of their blood volume will be taken. This will be done under anesthesia to minimise stress.	
4.	Asian elephant	*Elephas maximus*	Intravenous blood collection through auricular vein from 10 individuals. Less than 1% of their blood volume will be taken. This will be done under anesthesia to minimise stress.	
5.	Stray dogs	*Canis lupus familiaris*	Intravenous blood collection through cephalic vein from 10 individuals. Less than 1% of their blood volume will be taken. This will be done under anesthesia to minimise stress.	

LICENCE REF.NO. : JKM/MBS.1000-2/2 JLD. 20 (145)

DATE OF APPROVAL : 06 NOVEMBER 2024

6.	Rat	*Rattus sp.*	Intravenous blood collection through tail vein from 50 individuals. Less than 1% of their blood volume will be taken. This will be done under anesthesia to minimise stress.	Bornean Sun Bear Conservation Centre, Sepilok Orangutan Rehabilitation Centre **(BSBCC, Sabah Wildlife Dept., Sabah Forestry Dept.)**

Terms and conditions

(Section 8B(c) & 15 of Sabah Biodiversity Enactment 2000)

22 NOV 2024

1. This licence is valid from .....for a period of 12 months.
2. The User must apply for a professional research pass from the Sabah Immigration Department, at their own cost.
3. This licence is not transferable. The User must produce this licence for inspection by the Director or any person authorized by the Director.
4. The User must apply for an 'Export Licence of Biological Resources' from the Director, if the User intends to export any biological resources from this access.
5. All biological resources collected shall be taken to, stored and preserved at:
   Sabah Wildlife Health, Genetic, and Forensic Laboratory.
6. The User must submit a copy of dissertation and/or related publications to Sabah Biodiversity Council at the end of their research.
7. The User must submit a progress report to the Director for an extension of the Access Licence.
8. Application for patent or other intellectual property rights within or outside of Malaysia are subject to first obtaining the prior written consent of the Sabah Biodiversity Council.
9. The Principle Investigator and Local collaborator are both fully responsible for the conduct of all members named in the research group; to ensure they are only allowed within the permitted areas, and sample number/volume of biological resources taken are as approved by The Sabah Biodiversity Council.
10. The User must not use the biological resources or traditional knowledge associated with biological resources, to which the application relates, for commercial purposes.
11. The User must return one (1) identified set of the specimens to the Local Collaborator upon completion of the study; and must not distribute any part of the biological resources and/or specimens to a third party, other than disclosed in the Export Licence. Any intention to distribute the samples is subject to first obtaining the prior written consent of the Director.
12. The User must not allow others to carry out, research or development for commercial purposes on any genetic resources or biochemical compounds comprising or contained in the biological resources or traditional knowledge associated with biological resources unless a benefit-sharing agreement has been entered into.
13. Any offence committed under this Enactment shall be subject to offence under Section 26 of The Sabah Biodiversity Enactment 2000.

New: *X* Renewal:

(KEN KARTINA BINTI KHAMIS)
Director
of Sabah Biodiversity Centre